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Predominant emphysema phenotype in chronic obstructive pulmonary disease patients

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ABSTRACT: Patients with fixed airflow limitation are grouped under the heading of chronic obstructive pulmonary disease (COPD). The authors investigated whether COPD patients have distinct functional, radiological and sputum cells characteristics depending on the presence or absence of emphysema.

Twenty-four COPD outpatients, 12 with and 12 without emphysema on high-resolution computed tomography scan of the chest, were examined. Patients underwent chest radiography, pulmonary function tests and sputum induction and analysis.

Subjects with documented emphysema had lower forced expiratory volume in one second (FEV_1), FEV_1/forced vital capacity ratio, and lower carbon monoxide diffusion constant (K_{CO}), compared with subjects without emphysema. Chest radiograph score of emphysema was higher, chest radiograph score of chronic bronchitis was lower, and the number of sputum lymphocytes was increased in patients with emphysema, who also showed a negative correlation between K_{CO} and pack-yrs.

Chronic obstructive pulmonary disease patients with emphysema, documented by high-resolution computed tomography scan, have a different disease phenotype compared with patients without emphysema. Identification of chronic obstructive pulmonary disease-related phenotypes may improve understanding of the natural history and treatment of the disease.

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Chronic obstructive pulmonary disease (COPD) is characterised by the progressive development of airflow limitation that is not fully reversible [1]. Chronic airflow limitation is caused by a mixture of small airways inflammatory disease, obstructive bronchiolitis and parenchymal destruction, and emphysema, the relative contributions of which vary from person to person [2].

Chronic inflammation causes remodelling and narrowing of the small airways and, theoretically, it responds to pharmacological treatment. Destruction of the lung parenchyma leads to the loss of alveolar attachments to the small airways and decreases lung elastic recoil. These changes, which are considered to be unresponsive to pharmacological treatment, diminish the ability of the airways to remain open during expiration.

COPD has a variable natural history and not all individuals follow the same course and have the same response to therapy. Patients with emphysema have the lowest survival rate and the highest rate of decline in pulmonary function among COPD patients [3]. Thus, it is clinically relevant to detect, in the diagnostic work-up of patients with COPD, individuals with emphysema, possibly by using noninvasive methods. Although it has become increasingly clear that lung computed tomography (CT), especially when performed with thin-section, high-resolution techniques, is the most accurate imaging method for detecting the extent and severity of emphysema [4], lung function and chest radiography are invariably obtained in the clinical assessment of COPD. Recently, the application of sputum induction and refined methods of sputum examination have provided the opportunity to examine cell and molecular markers of airway inflammation in COPD [5], contributing to the characterisation of lung inflammation in the disease [6, 7].

The aim of this study was to investigate, by using classical diagnostic tests, such as lung function tests, radiological examination and sputum analysis, whether COPD patients with documented emphysema at high-resolution computed tomography (HRCT) of the chest have a different disease phenotype compared with COPD patients without emphysema.

Subjects and methods

Subjects

COPD patients (n=24) with fixed airflow limitation, *i.e.* forced expiratory volume in one second (FEV_1) <70% of predicted values and FEV_1/forced vital capacity (FVC) ratio <70%, were examined both before and after 200 μg of inhaled salbutamol (table 1). Chronic bronchitis was defined as cough

Table 1. – Characteristics of the chronic obstructive pulmonary disease (COPD) patients divided according to the presence or absence of emphysema on high-resolution computed tomography examination[#]

Characteristic/pulmonary function response	Emphysema	No emphysema
Age yrs	68±2	68±2
Sex M:F	11:1	11:1
Smoking history pack-yrs	29±6	36±7
Body mass index	28±1.4	26±0.8
Treatment		
Inhaled steroids n (%)	11 (91.6)	7 (58.3)
Inhaled bronchodilators n (%)		
Short-acting	9 (75)	7 (58.3)
Long-acting	10 (83.3)	8 (66.6)
Theophylline	4 (33.3)	0 (0)
Positive skin-prick test n (%)	2 (16.6)	4 (33.3)
FEV_1 % pred pre-bronchodilator	38±3[¶]	58±3
FEV_1/FVC % pre-bronchodilator	40.69±3.3[¶]	54.68±2.3
RV % pred	129±8	111±10
TLC % pred	98±4	95±4
IC % pred	78.6±4.5[¶]	104.2±4.8
FRC % pred	112±6*	95±7
*K*CO % pred	48±8*	71±7
ΔFEV_1 post-bronchodilator mL	120±25	141±28
ΔFEV_1 post-bronchodilator % pred	4.3±0.9	5.4±1.1

Data are presented as mean±SEM. M: male; F: female; FEV_1: forced expiratory volume in one s; % pred: % predicted; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; IC: inspiratory capacity; FRC: functional residual capacity; *K*CO: carbon monoxide diffusion constant. [#]: score >10 indicated emphysema. *: $p<0.05$ *versus* COPD patients with no emphysema; [¶]: $p<0.005$ *versus* COPD patients with no emphysema.

and sputum production occurring on most days of the month for at least 3 months per year, during the 2 yrs before the study [2]. All subjects were recruited from the outpatient clinic of the Section of Respiratory Diseases of the University of Ferrara, Ferrara, Italy. The Ethics Committee of the University Hospital of Ferrara approved the study and all patients gave their written informed consent. COPD was diagnosed according to the criteria recommended by the European Respiratory Society [8]. All patients were in stable conditions at the time of the study and free from acute exacerbations of symptoms and upper respiratory tract infections in the 2 months preceding the study.

Study design

Emphysema was characterised using HRCT technique and a visual score >10 indicated emphysema [9]. Each patient underwent medical history, physical examination, pulmonary function tests, chest radiography, CT, skin-prick tests, induced sputum, and α_1-antitrypsin measurement, since three of the patients were nonsmokers.

Pulmonary function studies

In each patient, lung volumes (Biomedin, Padova, Italy), the carbon monoxide diffusion constant (*K*CO), by the single-breath technique, end-exhaled nitric oxide (NO) (Nitric oxide A 280 analyser; Sievers, Boulder, CO, USA), arterial oxygen (P_aO_2) and carbon dioxide tension (P_aCO_2) (Instrumentations Laboratories, Milan, Italy) were measured as described previously [8], according to published guidelines [10–12].

Chest radiography

Chest radiographs (postero-anterior and lateral) were obtained with the patients in an upright position, holding their breath at full inspiration. A standard 2 m focus-to-film distance was used. Exposure time was kept as short as possible to reduce motion blurring and was usually within 0.05 s. A fine lead grid (grid ratio 6) was used to reduce scattered X-rays, thereby enhancing film resolution. Chest radiographs were obtained within 48 h of pulmonary function studies and prior to the sputum inductions. Chronic bronchitis and emphysema scores, ranging 0–16, were calculated as described previously [9, 13]. Emphysema and chronic bronchitis scores have proved highly reproducible among different observers [9, 13].

Computed tomography

HRCT scans were performed at suspended full inspiration on a TOMOS 7000 scanner (Philips, Einthoven, The Netherlands), as described previously [9]. No contrast medium was infused. Technical parameters were 1 mm collimation, 120 kV peak, 160 mA, and 3-s scanning time. A visual score of emphysema was derived according to the method of SAKAI *et al.* [14] and a cumulative score of emphysema, ranging 0–72 was obtained, as described previously [9].

Induced sputum

Sputum was collected after bronchodilator inhalation and analysed as described previously [8]. To make the procedure safer, sputum was induced and spirometric measurements were performed as reported previously [15]. Increasing concentrations of hypertonic saline were nebulised with an ultrasonic nebuliser (Mistogen EN 145 electronic nebuliser; Mistogen Equipment Co., Oakland, CA, USA).

Atopic status

Skin-prick tests to 12 common aeroallergens were performed according to a standard protocol [8].

α_1-antitrypsin

A peripheral blood sample was drawn and the serum was separated. Antibodies against human α_1-antitrypsin were added to the serum and the intensity of the light diffused by suspended particles, resulting from the formation of the antigen/antibody complex, was measured with a nephelometric method (Beckman Instruments, Milan, Italy).

Statistical analysis

Group data are expressed as mean±SEM or as median and interquartile range when appropriate. Differences between groups were analysed using the Mann-Whitney U-test and Bonferroni's correction was applied when indicated. Categorical values were analysed using Fisher's exact test. Spearman's rank correlation test was used to examine the association between lung and radiological data and cigarette smoking and/or sputum cells. To define the predictive value of the variables analysed, receiver-operating characteristic (ROC) curve analysis was performed for a comparison of ranked variables. The area under the ROC curves was determined and a value of >0.80 was accepted as indicating good discrimination [16]. ROC curve analysis also allowed selection of the best cut-off

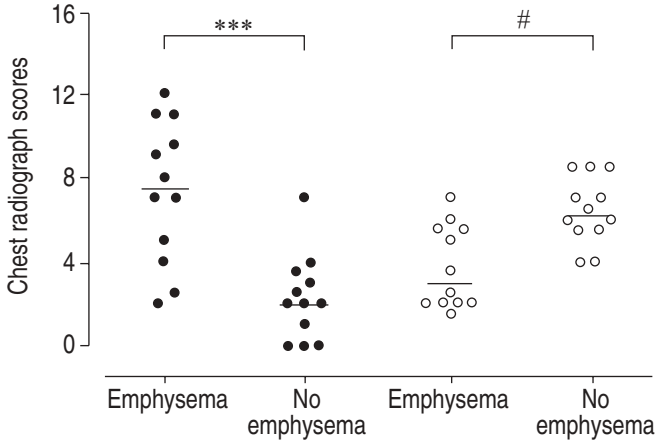

Fig. 1. – Chest radiograph scores of emphysema (●) and chronic bronchitis (○) in chronic obstructive pulmonary disease patients, divided according to the presence or absence of emphysema. The horizontal solid bars indicate the median values for each group. ***: $p<0.001$; #: $p<0.005$.

point of the variables studied by analysing their sensitivity *versus* one minus specificity. A p-value of <0.05 was accepted as significant.

Results

Clinical findings

Table 1 shows the characteristics of the subjects examined. There was no significant difference in age, smoking history and body mass index between COPD patients with emphysema and patients with no emphysema. In all subjects, α_1-antitrypsin values were within the normal range (83–199 mg·dL^{-1}). Dyspnoea, graded according to the Medical Research Council dyspnoea scale [17], was mild and similar in the two groups. Three of the 12 COPD patients with and eight of the 12 without emphysema had symptoms of chronic bronchitis.

Pulmonary function findings

There was no significant difference in total lung capacity (TLC; % pred), exhaled NO, arterial blood gases and response to inhaled salbutamol between the COPD patients with and without emphysema (table 1). Emphysema patients had a significantly lower FEV1 (% pred), FEV1/FVC ratio (%) and *K*CO (% pred) than patients with no emphysema. For similar values of TLC in the two groups, inspiratory capacity (IC; % pred) was significantly lower, whereas functional residual capacity (FRC; % pred) was significantly higher in COPD patients with emphysema, indicating greater pulmonary hyperinflation. Residual volume (RV; % pred) was higher ($p=0.09$) in the emphysema group. In this group, a negative correlation was found between *K*CO and cigarettes smoked ($r=-0.83$; $p<0.01$).

Chest radiography

As expected from the selection criteria, COPD patients with emphysema had a significantly higher chest radiograph score of emphysema (7.5 (4.5–10) *versus* 2 (0.5–3), $p<0.001$; fig. 1) and a lower chest radiograph score of chronic bronchitis (3 (2–5.5) *versus* 6 (5.5–5.7); $p<0.005$; fig. 1) than patients with no emphysema.

Table 2. – Sputum cells in chronic obstructive pulmonary disease (COPD) patients divided according to the presence or absence of emphysema on high-resolution computed tomography examination

Cells	COPD patients with emphysema	COPD patients with no emphysema
Total nonsquamous cells $1\times10^4\cdot mL^{-1}$	48 (23–111)	60 (17–359)
Macrophages %	30.6 (15.0–46.3)	10.2 (4.7–28.0)
Neutrophils %	64.9 (49.6–82.0)	83.6 (65.5–91.4)
Eosinophils %	2.4 (1–3)	2.5 (1.2–6.3)
Lymphocytes %	1.5 (0.7–3.5)*	0.2 (0–1.0)

Data are presented as median (interquartile range). *: $p<0.05$ *versus* COPD patients with no emphysema.

Sputum findings

Patients with emphysema had more sputum lymphocytes than patients with no emphysema. No other significant differences were observed (table 2). When all COPD patients were considered together, sputum lymphocytes correlated negatively with IC % pred ($r=-0.46$, $p<0.05$) and positively, albeit not to a significant extent, with the HRCT score of emphysema ($r=0.40$, $p=0.06$). In emphysematous patients, a positive correlation was found between the HRCT score of emphysema and sputum neutrophils ($r=0.60$, $p<0.05$; fig. 2).

Receiver-operating characteristic curve analysis

FEV1, chest radiograph score of emphysema and sputum lymphocytes were shown to be the best predictive factors of emphysema. For FEV1 % pred, the area under the ROC curves was 0.89, for emphysema score it was 0.90, and for the percentage of lymphocytes in sputum it was 0.81. For FEV1 % pred, the best cut-off point was 49% pred, which had a sensitivity of 0.83 and a specificity of 0.83. For the chest radiograph score of emphysema, the best cut-off point was 4.0, which had a sensitivity of 0.75 and a specificity of 0.80. For sputum lymphocytes, the best cut-off point was 1%, which had a sensitivity of 0.67 and a specificity of 0.83.

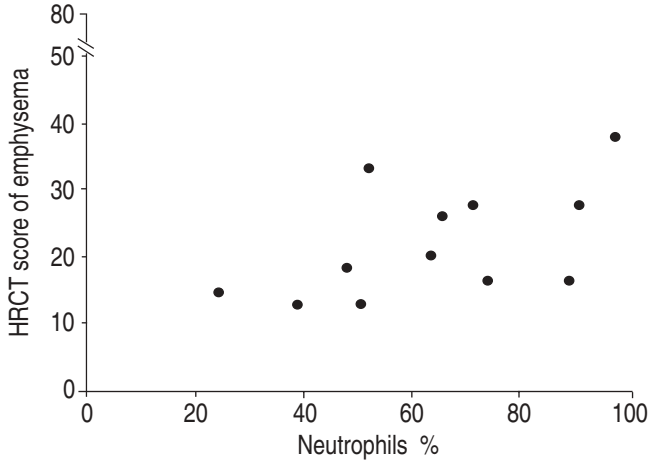

Fig. 2. – Relationship between high-resolution computed tomography (HRCT) score of emphysema and percentage of sputum neutrophils (Spearman's rank correlation: $p<0.05$, $r=0.60$) in chronic obstructive pulmonary disease patients with emphysema.

Discussion

In this study, the authors have shown that patients with fixed airflow limitation (COPD) have a different disease phenotype, depending on the presence or absence of emphysema on chest HRCT. Patients were selected on the basis of functional criteria, *i.e.* FEV1 <70% pred and FEV1/FVC ratio <70% both before and after inhaled bronchodilator. These inclusion criteria were used as they define most of the COPD patients involved. COPD subjects with emphysema had lower FEV1, lower FEV1/FVC ratio, lower *K*CO, higher chest radiograph scores of emphysema, lower chest radiograph scores of chronic bronchitis, and an increased number of lymphocytes in induced sputum.

These findings of a higher degree of airflow obstruction in patients with emphysema are in agreement with the results of previous studies that focused on the relationship between severity and extent of emphysema and lung function data [18–22]. By contrast, these findings may appear to conflict with those of FLETCHER *et al.* [23], who found no differences in clinical and lung function features between emphysema and chronic bronchitis patients. However, they examined a different population: patients were younger, 20% exhibited reversible airflow limitation after inhaled bronchodilator, and the majority had a history of wheezing.

In the present study, a reduction in *K*CO was found in COPD patients with emphysema. The reduction of CO diffusing capacity is a physiological abnormality known to be associated with pulmonary emphysema, and it has been shown to correlate with the extent of emphysema [24]. In emphysematous patients, a negative correlation between *K*CO and pack-yrs was also observed, which supports and extends the findings of other studies [25, 26] that have shown a negative correlation between lung function and pack-yrs even in asymptomatic subjects [26]. The emphysematous subjects in this study exhibited baseline values of IC and FRC smaller and greater, respectively, than those of patients with no emphysema. As baseline TLC was not different between the two groups, these data reflect a greater pulmonary hyperinflation in emphysematous patients. In these patients, RV and TLC were higher, but not significantly so, thus indicating that subjects with emphysema do not have a pure emphysema phenotype and, therefore, lack the fairly uniform pattern of abnormal pulmonary function tests characteristic of emphysema. The lack of significant differences in these variables, however, does not exclude meaningful differences.

In the present COPD patients, the distinction between emphysema and no emphysema on the HRCT was based on a cut-off of 10 of the applied visual score, which ranged from 0–72. According to previous findings [9], the authors were confident that an HRCT score of emphysema that did not exceed 14% of the maximal score obtainable, was compatible with no or with only trivial emphysema.

With regard to imaging tests, it has been demonstrated that conventional chest radiography is useful in the clinical evaluation of emphysema [9]. The fact that, in this study, a higher chest radiograph score of emphysema and a lower score of chronic bronchitis were present in patients with emphysema, confirms that this examination is useful to discriminate the two phenotypes, *i.e.* emphysema and no emphysema. When all the patients were considered together, chest radiograph scores of chronic bronchitis showed a negative correlation with the HRCT emphysema score (data not shown), suggesting that the features of bronchial and/or bronchiolar inflammation are predominant in patients with no emphysema. In this study, symptoms of chronic bronchitis were present in the majority of patients without emphysema, but only in three subjects with emphysema, confirming that hypersecretion of mucus is present more in patients with no or trivial emphysema [22]. Three subjects without emphysema on HRCT scan and without symptoms of chronic bronchitis were also observed. These subjects had a high radiograph score of chronic bronchitis, 6.5, 5.5 and 8.5 (scores ranging 1.5–8.5), suggesting that chest radiography detects bronchial and/or bronchiolar inflammation better than the symptoms. Another possible explanation for this lack of relationship between symptoms and chest radiograph is a different time course for the two events, with early radiological changes later followed by symptoms.

This study, using induced sputum, demonstrated a slight but significant increase in the number of sputum lymphocytes in patients with emphysema. No differences were observed in other sputum inflammatory cells, supporting the evidence that these patients did not have a pure component of lung parenchymal destruction, *i.e.* a pure emphysema phenotype, and also an airway inflammatory component. Lymphocytes, particularly CD8+ cytolytic T-cells, have been recognised recently as the predominant cells in the alveolar wall of smokers with emphysema [27]. The results, obtained by a noninvasive method, such as sputum induction, confirm this finding. Moreover, a negative correlation was found between sputum lymphocytes and IC, which typically decreases in emphysema due to pulmonary hyperinflation. Taken together, all the above data suggest that lymphocytes are the inflammatory cells best associated with emphysema and they may be obtained by sputum induction. Therefore, it could be useful to introduce this noninvasive method into the regular work-up of COPD patients, given that it is also simple and safe [28]. Finally, regarding sputum differential cell counts, it should also be noted that there was a correlation between the HRCT score of emphysema and the number of neutrophils. This finding seems to support the hypothesis that neutrophils are implicated in the severe stage of COPD, as other studies have also suggested [29, 30].

Although in COPD patients the two components of airflow limitation, small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), often coexist, it could be clinically relevant to distinguish subjects with predominant emphysema phenotype.

In conclusion, the authors showed that forced expiratory volume in one second, chest radiograph score of emphysema and sputum lymphocytes are the three indices that better distinguish emphysema from nonemphysema patients. Such distinction could be useful in clinical practice, may improve understanding of the natural history of the disease, and may help to focus treatment strategies for different chronic obstructive pulmonary disease phenotypes, as, for example, the more the lung destructive component is widespread, the less anti-inflammatory drugs are expected to be of benefit.

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